Expression of Mgp in Calcification Leiomyoma uterus in postmenopausal women

Ekram Saad Abd Al Hussein Al Gharibawi[1], Ahmed Mahdi Saleh Al-Badri[2], Faris Lutfi Nussrat[3]

[1]*College of Science, University of Wasit, Iraq*
[2]*College of Science, University of Wasit, Iraq*
[3]*Al-Karamah Teaching Hospitals, Wasit Governorate, Iraq*

KEYWORDS
Calcification, MGP, fibroid, leiomyoma

ABSTRACT
The Matrix Gla protein (MGP) is an extracellular polypeptide that can inhibit calcification in tissues that express Mgp molecules. An analysis employing histology and immunohistochemistry unveiled the general architecture and detected calcification within the fibroid. The study included gathering twelve samples after confirming through histological examination that the samples were affected by fibroid cysts. Each of these specimens was conserved in a 10% formalin solution. A histological examination was performed using two dyes, hematoxylin and eosin, to demonstrate the general histological organization. The Van Kossa stain was employed to detect the buildup of calcium anions in the tissue. Moreover, the immunohistochemical technique was used to detect the elevated expression of Mgp in the myofibers of leiomyoma-affected myometrium tissue.

1. Introduction

Uterus is the vital reproductive organ of female which is hormone responsive[1]. Myometrium is the thick, smooth muscle coat of the uterus underneath the endometrium and is covered by the peritoneum derived serosa[2]. Among diverse benign lesions of Myometrium, leiomyoma is the commonest visceral neoplasm affecting females in reproductive age group[3]. They are the chief cause for hysterectomy all over the globe; followed by adenomyosis, leiomyosarcoma, endometrial stromal tumors, secondary tumors and vascular lesions etc [4].

The histopathological features of fibroid as a benign uterine pathology shows great variability as regards to clinical presentation, site, number and presence of degenerative changes[5,6]. Calcification is the biochemical process by which calcium salts accumulate in bodily tissues. The phenomenon often arises during bone formation, yet, there can be aberrant deposition of calcium in soft tissue, leading to its hardening [7]. Factors such as age, vitamin D, and high levels of calcium intake are key determinants of parathyroid hormone-induced calcification development [8]. The majority of uterine calcifications in women are benign. Calcification is the final step of hyaline degeneration in uterine fibroids, characterized by a distinctive center popcorn pattern. Contrarily, calcifications in treated fibroids following uterine artery embolization form in a smooth peripheral pattern, most likely due to the presence of polyvinyl alcohol particles in the peripheral fibroid arteries [9].

Among the proteins involved in modulating vascular calcium metabolism, it has been hypothesized that the vitamin K-dependent matrix Gla- (γ-carboxyglutamate) protein (MGP) plays a dominant role.
.
The principal protein active in vascular calcium metabolism is the vitamin K-dependent matrix Gla-protein (MGP). The general population has been subjected to characterization of MGP species in both tissue and the bloodstream. Significant changes have been detected in individuals who have been diagnosed with cardiovasculardisease(CVD)[10].

Matrix Gla-protein (MGP) is a compact secretory protein capable of undergoing two forms of further modifications after translation: γ-glutamate carboxylation and serine phosphorylation. The protein was initially documented in 1983 by Price et al., who isolated it from the bone matrix of bovine sheep [11].

. MGP is a local natural calcification inhibitor secreted primarily by chondrocytes and vascular smooth muscle cells in the arterial tunica media [12,13, 14). MGP acts as a strong inhibitor of soft

tissue calcification [15]. To acquire its full calcification inhibitory activity, MGP needs to undergo two post-translational modifications: glutamate carboxylation and serine phosphorylation. Both modifications are not exerted completely, so theoretically, four different MGP conformations can be found: unmodified and inactive as dp-ucMGP, only phosphorylated,only carboxylated, and finally fully modified and active as phosphorylated and carboxylated MGP. In essence, high levels of plasma dpucMGP are a proxy for vitamin K deficiency [12, 13,14]. This, we aimed in this paper to observe Mgp expression in leiomyoma uterus In case of tissue calcification

2. Material and method

Samples collection

Twenty uterine fibroid tissue samples were collected, either after hysterectomy or myomectomy, aged (40-50) years, at Al-Karamah Teaching Hospitals in Wasit Governorate. Preserved in a 10% formalin solution, the specimens were subjected to several histological procedures :dehydration, disinfection, infiltration, embedding, sectioning, and staining with hematoxylin and eosin (H&E) stain to reveal the pathological condition of the tissue[16]. Additionally, Von Kossa stain was used to identify calcium salt deposits in paraffin sections.

Immunohistochemistry technique

Briefly, charge slides containing wax embedded uterine tissues were dewaxed in 100% toluene for 30 minutes. The tissue was immersed in a decreasing concentration of absolute ethanol for 10 minutes, followed by further immersions in 90% and 70% ethanol for one minute each. Thoroughly rinsed slides 2-3 times with clean water. The method should be grounded on the production process depending on the manufacturer, where slides are submerged in a citrate buffer solution and then heated in an oven to 100 ºC to facilitate antigen retrieval. After incubating for 30 minutes, the slides were cooled to room temperature (RT). Thoroughly rinsed slides with phosphate buffer saline (PBS) three times. The sections were treated with a protein blocking solution for 10 minutes at room temperature. Slides were treated with the primary antibody MGP Antibody (H-4)sc-271907 SantaCruz/USA for 30 minutes at room temperature. Further, the slides were rinsed with PBS seven times. The slides were treated with a one-step HRP polymer for 30 minutes at room temperature. The slides were rinsed seven times with PBS and further three times with distilled water. Drop small amounts of DAB reagent over tissue slides and allow 10 minutes at room temperature. Each slide was rinsed seven times with PBS and then rinsed again with distilled water. Next, the slices were treated with hematoxylin for 60 seconds. Afterwards, the slides were rinsed with distilled water until they became transparent, then embedded with D.P.X., a mounting media, and covered with a coverslip.

3. Result and Discussion

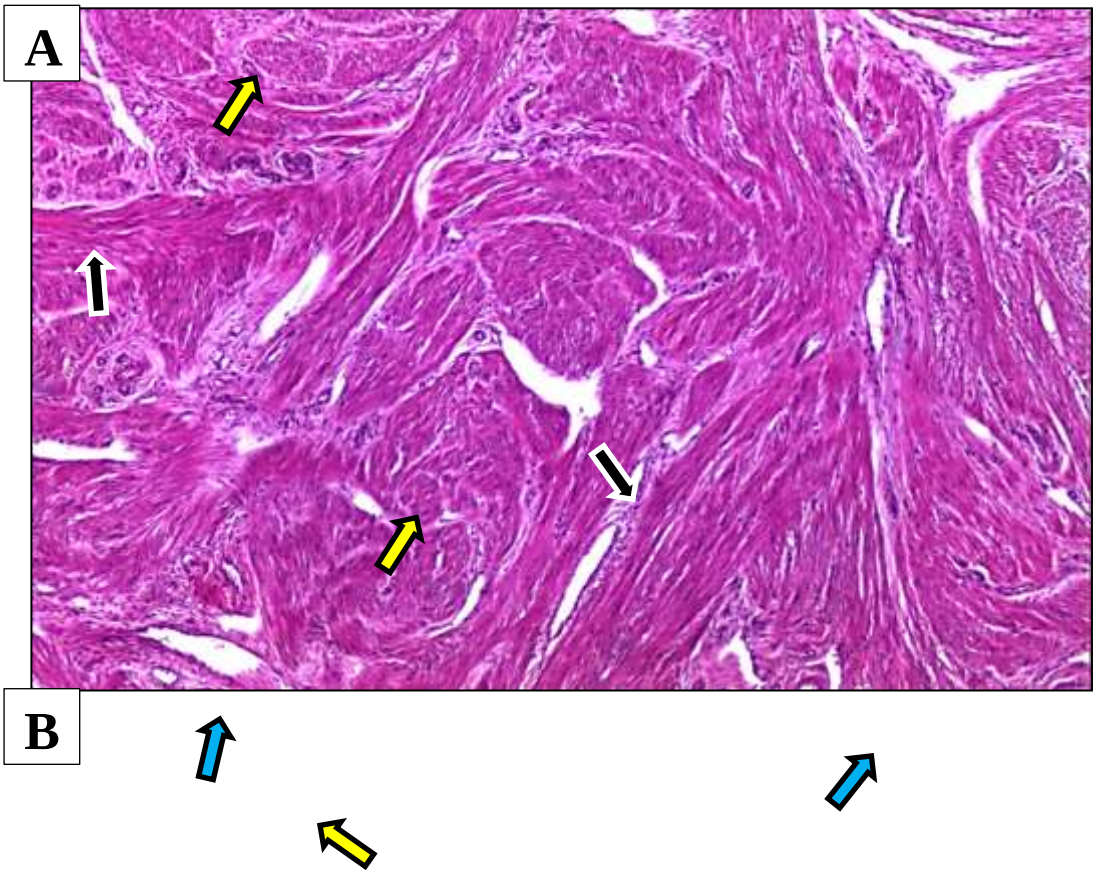
Histological and Immunohistochemical results

The histological findings of the standard staining (hematoxylin and eosin) disclosed an excessive proliferation of fibrous connective tissue. This fibrous tissue often exhibits a high density and possesses distinct structural features compared to the typical myometrium. The fibrous tissue exhibited an uneven arrangement, resulting in the development of a firm mass or nodule within the myometrium. These tissue alterations encompass fibrosis and the buildup of collagen and connective fibers, resulting in the loss of tissue elasticity and the development of fibrosis. In fibrosis, histological alterations involve the buildup of collagen and connective fibers, resulting in tissue stiffening and the loss of the typical flexibility of the myometrium (Figure1). This finding is consistent with [17]. who observed that the histologic characteristics of Lamellar myometrium (LM) are consistently distinct from those of the surrounding normal myometrium. The majority of leiomyomas have a significantly higher amount of extracellular matrix deposition compared to normal myometrium. They manifest as aggregated masses of smooth muscle cells that are rich in collagen. LM usually contains 50% more collagen than normal myometrium and a higher percentage of type I collagen. The 4th edition of the World Health Organization Classification of tumors (Tumors of the Breast and Female genital

organs) defines leiomyomas as benign neoplasms consisting of disordered smooth-muscle cells that are buried in large amounts of extracellular matrix. This diseases is characterized by alterations in the structure and cellular morphology of the myometrium. Descriptively, this disease is characterized by its numerous, spherical shape, firmness, pale to tan color, and whorled trabecular texture. This non-malignant tumor induces distortion of adjacent tissues, frequently accompanied by degenerative alterations [18].

Upon microscopic analysis, leiomyomas have spiral, linked cellular formations that can be either uni- or fusiform. Typically, cells display a spindle-shaped morphology, a large fibrillar assembly, cytoplasm that is eosinophilic, and indistinct borders. In certain cases, particularly in cellular leiomyomas, the cytoplasm is sparse and the arrangement of cells into fascicles may be lacking. Small nucleoli and finely dispersed chromatin are the defining features of nuclear structures. Geolocally located leiomyomas display a greater level of cellularity in comparison to the adjacent myometrium [19].

Histological analysis utilizing calcium detecting stains (von Kossa stain) the black to dark brown color-stained tissue, indicating the deposition of calcium in myofibers tissue of the affected myometrium (Figure2). Immunohistochemical finding using the primary antibody MGP Antibody (H-4)sc-271907 from Santa Cruz, USA, revealed an overexpression of Matrix Gla protein (MGP) in the myofiber of leiomyoma-affected myometrium(Figure3). This result is in agreement with However, both forms of MGP accumulate in hardened atherosclerotic lesions and in Mönckeberg's media sclerosis, likely due to heightened expression. Reports indicate that increased calcium levels stimulate the production of MGP in cultures of vascular smooth muscle cells [20].



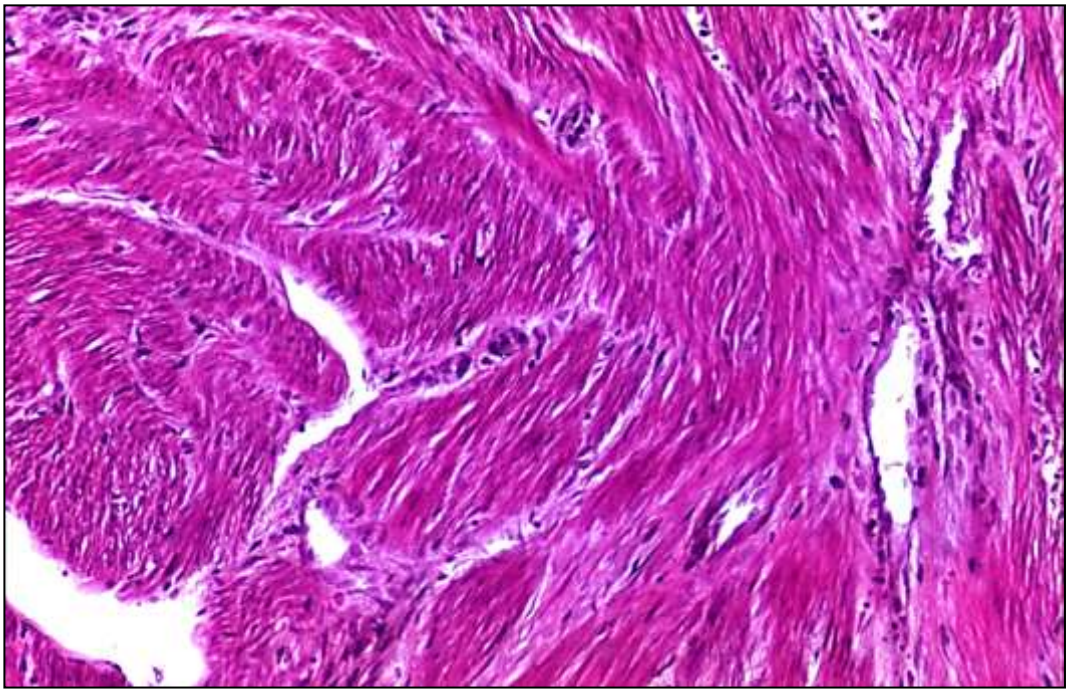


Figure 1: Photomicrograph of the uterus of 50-60 years-old woman

A&B/ Uterus Fibroid (leiomyoma). Note the proliferation of new myocytes formed irrgular myofibers, where the myofibers showed the variation in its directions. Where, the some myofibers showed longtudenal direction (black arrow) and other showed transvers direction (yellow arrow) in same muscular tissue, also the increasing of fibrous tissue (blue arrow) between the myofibers was observed in the affected area. **H&E. A: 40x and B: 100x.**

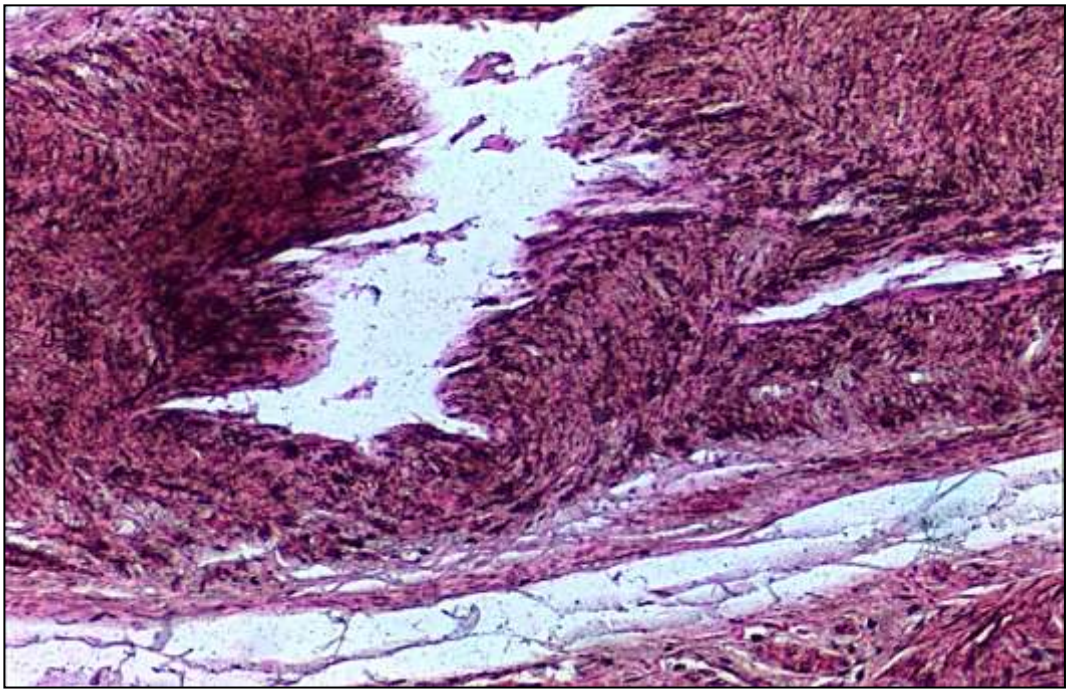
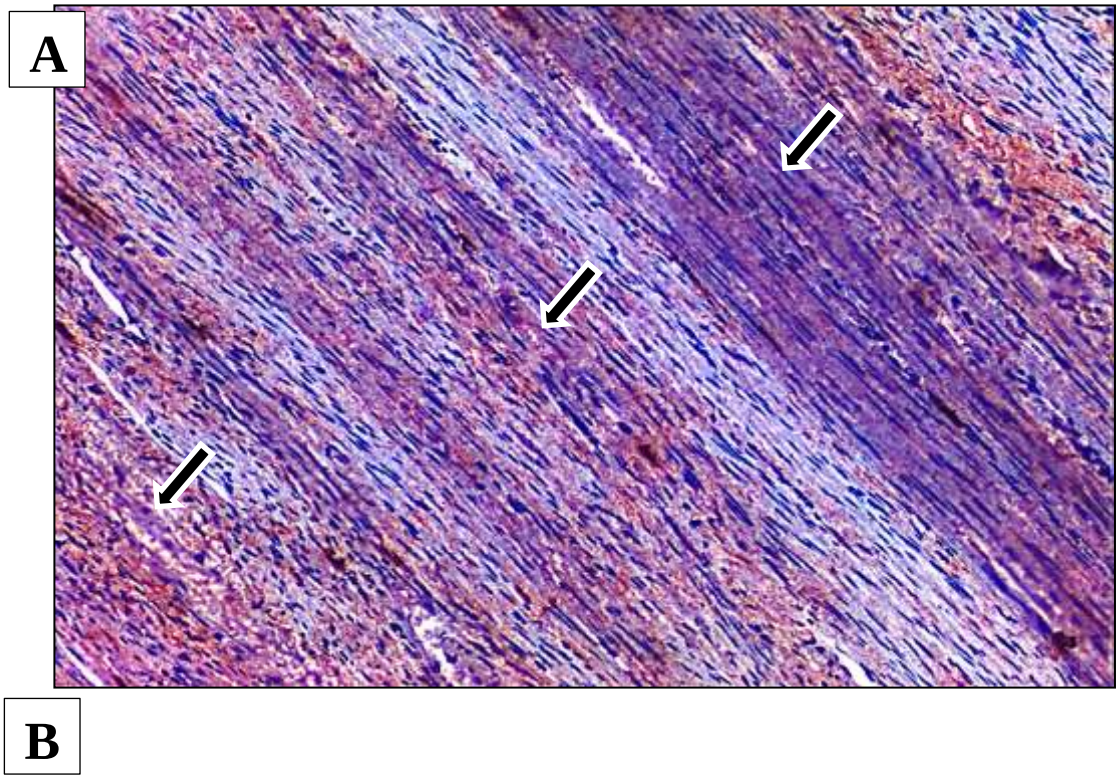


Figure 2: Photomicrograph of the uterus of 50-60 years-old woman.

A&B/ Uterus fibroid. Note the black to dark brown color-stained tissue, indicating the deposition of calcium in myofibers tissue of the affected myometrium. However, the calcium deposition was observed significantly, in certain the muscular tissue area (black arrow) that surrounded by fibrous capsule (yellow arrow). **Von Kossa stain. A: 40x and B: 100x.**



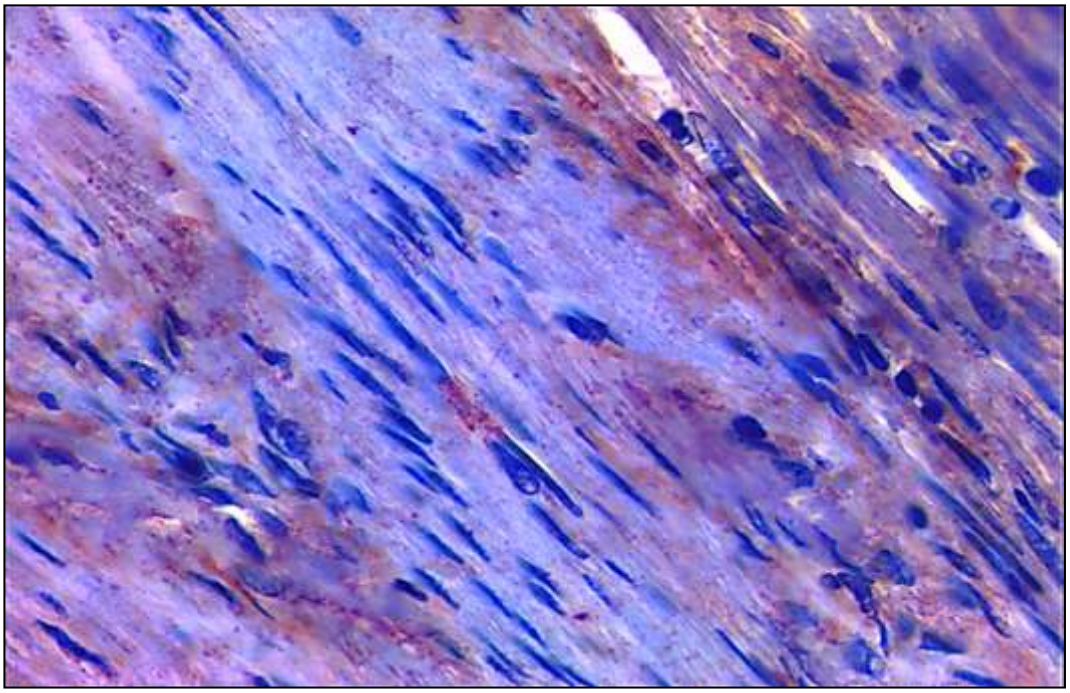


Figure3: Photomicrograph of uterus of 50-60 years-old woman

A&B/ Uterus fibroid (leiomyoma). Note the overexpression of Matrix Gla protein (MGP) within the myofibers of leiomyoma-affected myometrium tissues. Notably, this overexpression was significant in the longitudinally arranged myofibers (black arrow). **DAB and Hematoxylin. A: 100x and B: 400x.**

Conclusion

Histologically, hematoxylin and eosin stain showed an overgrowth of fibrous connective tissue. This fibrous tissue usually appears dense and has different structural characteristics than normal myometrium. Van Kosa stain shows black to dark brown stained tissue suggesting calcium deposition in the muscle fibers of affected myometrium, and immunohistochemical technique showing overexpression of Mgp due to deposition of high levels of calcium. in myofiber tissue of the affected myometrium. This overexpression was significant in the longitudinally arranged myofibers.

Reference

[1] Crum CP. Body of uterus and endometrium: Kumar V, Abbas AK, Fausto N, Eds. Robbins and Cotran Pathologic Basis of Disease 7th ed. Philadel-phia: Saunders. 2004;1089-90.

[2] Christopher PC. The female genital tract: Kumar, Abbas, Fauster eds. Robbins and Cottron Pathologic Basis of Disease 8th.

[3] Melmed S, PolonskyKS, Larsen PR, et al. Williams textbook of endocrinology 13th ed. Philadelphia: Elsevier 2016.

[4] Silverberg SG, Tabbara SO. The uterine corpus. In: Silverberg SG, Delellis. RA, Frable WJ, Eds. Principles and Practice of Surgical Pathology and Cytopathology. Vol 3 (3rd edition). New York: Churchill Livingstone. 1997;2459-516.

[5] Sparic R, Mirkovic L, Malvasi A, et al. Epidemiology of uterine myomas: a review. Int J FertilSteril. 2016;9:424

[6] Di Tommaso S, Massari S, Malvasi A, Vergara D, Maffia M. Selective genetic analysis of myomapseudocapsule and potential biological impact on uterine fibroid medical therapy: 2015.

[7] Miller, J. D. (2013): Cardiovascular calcification: Orbicular origins. Nature .Materials 12, 476-478.

[8] Perry,T.W. (1984): Animal Life-Cycle Feeding and Nutrition. Academic Press,

[9] INC.New York, USA. Pp: 22- 27.

[10] Nicholson TA, Pelage JP, Ettles DF. Fibroid calcification after uterine artery embolization: ultrasonographic appearance and pathology. J Vasc Interv Radiol 2001;12(4):443–446.

[11] Leon J. Schurgers , Ellen C. M. Cranenburg , Cees Vermee Matrix Gla-protein: The calcification inhibitor in need of vitamin K Thromb Haemost 2008; 100(04): 593-603

[12] Price PA, et al. Matrix Gla protein, a new gammacarboxyglutamic acid-containing protein which is associated with the organic matrix of bone. Biochem Biophys Res Commun 1983; 117: 765–771.

[13] Schurgers, L.J., Barreto, D.V., Barreto, F.C., Liabeuf, S., Renard, C., Magdeleyns, E.J., Vermeer,C., Choukroun, G., Massy, Z.A., 2010. The circulating inactive form of matrix Gla protein is a surrogatmarker for vascular calcification in chronic kidney disease: a pre liminary report. Clin. J. Am. Soc. Nephrol. 5, 568–575.

[14] Liu, Y.P., Gu, Y.M., Thijs, L., Knapen, M.H.J., Salvi, E., Citterio, L., Petit, T., Delli Carpini, S.,Zhang, Z., Jacobs, L., Jin, Y., Barlassina, C., Manunta, P., Kuznetsova, T., Verhamme, P.,Struijker-Boudier, H.A., Cusi, D., Vermeer, C., Staessen, J.A., 2015. Inactive matrix Glaprotein is causally related to adverse health outcomes: a Mendelian randomization study in a Flemish population. Hypertension 65, 463–470 (1).

[15] Wei, F.F., Drummen, N.E., Schutte, A.E., et al., 2016. Vitamin K dependent protection of renal function in multi-ethnic population studies. EBioMed. 4, 162–169.

[16] Luo, G., Ducy, P., McKee, M.D., Pinero, G.J., Loyer, E., Behringer, R.R., Karsenty, G., 1997.Spontaneous calcification of arteries and cartilage in mice lacking matrixGLA protein. Nature 386, 78–81.

[17] Bancroft JD, Suvarna KS, Laytonn C, (2018). Bancroft's Theory and Practice of Histological Techniques. Established itself as the standard reference for histo-technologists and laboratory scientists, p96

[18] Brosens I.: Uterine Leiomyomata: Pathogenesis and Management. Abingdon, England, Informa Healthcare/Taylor & Francis, 2006.

[19] Ciarmela P., Islam S., Reis F., Gray P.C., Bloise E., Petraglia F., et al.: Growth factors and myometrium: Biological effects in uterine fibroid and possible clinical implications. Human Reproduction Update.2011; 17 (6): 772–790.

[20] Tavassoli F., Schnitt S., Hoefler H., Boecker W., Rosai J., Heywang-Kobrunner S.H., et al.: Intraductal proliferative lesions. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. 2003; Vol. 4

[21] Mendoza FJ, Martinez-Moreno J, Almaden Y, Rodriguez-Ortiz ME, Lopez

[22] I, Estepa JC, Henley C, Rodriguez M, Aguilera-Tejero E. Effect of calcium

[23] and the calcimimetic AMG 641 on matrix-Gla protein in vascular smooth

[24] muscle cells. Calcif Tissue Int. 2011;88:169–78.